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Further characterization of Escherichia coli brain microvascular endothelial cell invasion gene ibeA by deletion, complementation, and protein expression.

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The ibeA gene (ibe10) previously identified by TnphoA mutagenesis is part of a 50-kDa full-length open-reading frame (ORF) encoded by a 1.37-kb DNA fragment. An isogenic in-frame deletion mutant of ibeA (ZD1) was constructed by chromosomal gene replacement with a suicide plasmid pCVD442 carrying a 2.1-kb DNA fragment with an ibeA deletion. Similar to the previously described TnphoA insertion mutant of ibeA, the isogenic ibeA deletion mutant ZD1 was significantly less invasive in human brain microvascular endothelial cells (BMECs) than the parent strain. The mutant ZD1 was fully complemented by the ibeA ORF. The ibeA gene was subcloned into pET28a(+) and was expressed as a recombinant protein with an N-terminal histidine tag. The recombinant IbeA protein had much greater activity (50 times) in blocking the invasion of BMECs by Escherichia coli K1 than did the partial protein fragment, which provides further evidence that ibeA is an important determinant for E. coli K1 invasion of BMECs.

PMID: 11237832 [PubMed - indexed for MEDLINE]

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